Influence of *Bacillus thuringiensis* var. *israelensis* on oviposition of *Aedes albopictus* (Skuse)

Craig A. Stoops

Department of Entomology, Clemson University, Clemson, SC 29634-0365, U.S.A.

Received 16 April 2004; Accepted 27 August 2004

ABSTRACT**:** The effect of *Bacillus thuringiensis* var *israelensis* (B.t.i.) on the oviposition behavior of *Aedes albopictus* was evaluated in the field and laboratory in Clemson, SC, U.S.A. In the field, water taken from containers in which mosquito larvae were reared (conditioned water) was placed in 16 containers. Eight containers received 50 µl of B.t.i., and eight with water only were kept as controls. In the laboratory, field-collected females of *Ae. albopictus* were placed in rearing chambers and provided two containers for oviposition, one with 50 µl of B.t.i and one a control with water only. Eight cage experiments were conducted, five using filtered tap water and three with conditioned water. In the field over the 13 trials, more eggs were laid in the containers with B.t.i. although no significant difference was found in the number of eggs between the treatment and controls over 72 h. In the laboratory, more eggs were laid in the containers with B.t.i. versus the controls. The containers had filtered tap water and B.t.i. had significantly more eggs laid in them compared to the controls**.** ***Journal of Vector Ecology*** **30 (1): 41-44. 2005.**

*Keyword Index***:** *Aedes albopictus*, oviposition, *Bacillus thuringiensis* var. *israelensis*, attractant, repellent.

INTRODUCTION

Aedes albopictus (Skuse) is an important pest mosquito and potential vector of dengue in the Southeastern United States (Moore et al. 1988). Because *Ae. albopictus* larvae are found in both natural and artificial containers, control programs focus on source reduction rather than insecticides. Insecticides are usually avoided for larval control because potentially thousands of containers must be found and treated, making this an extremely labor-intensive undertaking (Nasci et al. 1994). Despite this apparent drawback, several insecticides including *Bacillus thuringiensis* var. *israelensis* (B.t.i.) can be effective. B.t.i. is environmentally safe and efficient at killing mosquito larvae, and when used in container-breeding mosquito control programs it can reduce the overall number of biting females (Nasci et al 1994). To find a way to use this insecticide effectively, the use of liquid formulations of B.t.i. dispersed by Ultra Low Volume (ULV) technology have been tested to help manage populations of container-breeding mosquitoes (Lee et al. 1996, Yap et al. 1997). Tests in Malaysia found that B.t.i. applied by ULV at 0.3 L/min penetrated tires up to 30 m away and resulted in 60 – 100% mortality of *Aedes aegypti* larvae (Lee et al. 1996). In an adult and larval cage experiment, B.t.i. combined with pyrethroids dispersed as ULV spray provided 95% control for *Ae. aegypti* (Yap et al. 1997). Establishing long-term campaigns to reduce the source of container-breeding mosquitoes is difficult, but the distribution of B.t.i. via ULV could provide a time-efficient way to control larvae when outbreaks of diseases such as dengue occur.

As with any strategy for controlling larval mosquitoes with an insecticide, all of the habitat used by the mosquitoes may not be reached. A ULV application of B.t.i. may result in a mosaic of containers that receives doses ranging from lethal to zero. Even if these larvicide applications were combined with an adulticide, females would still be available to recolonize the containers. Female mosquitoes that have a choice of containers with B.t.i. in various concentrations might be able to detect the B.t.i. and either be attracted to or repelled from treated containers. Over time, selection might favor females that can detect and avoid containers with B.t.i., thereby limiting the success of a control program.

To determine if B.t.i. has an effect on ovipositing container-breeding mosquitoes, a field and laboratory experiment was conducted in Clemson, SC. Currently there is no organized control program for container-breeding species in Clemson, but lack of exposure to B.t.i cannot be assumed. Background levels of B.t.i. in the environment are unknown (Martin and Travers 1989). Even without direct selection by B.t.i., behaviors might exist that limit oviposition in lethal environments. Perhaps the B.t.i. toxins or surfactants added to the B.t.i. formulation might repel ovipositing females.

The aim of this research was to determine if *Aedes albopictus* detects and avoids laying eggs in containers with a level of B.t.i. that is lethal to larvae.

MATERIALS AND METHODS

The study was conducted on the grounds of the Clemson University Cherry Farm Insectary (N 34º 39.080', W 82º 50.186'), in an open area that was adjacent to equipment storage buildings and a small deciduous forest. A complete randomized block design was used for the field experiment.

Present address: Navy Medical Research Unit 2, U.S. Embassy Jakarta Indonesia, Unit 8132 NAMRU 2, FPO AP 96520-8132

The experiment used the row as the block. Sixteen ovitraps were placed in a grid of four rows of four ovitraps, with 30 cm separating each ovitrap. Each ovitrap consisted of a 9.5 cm x 8.5 cm black plastic seedling pot that contained a 296 ml clear plastic cup and a 9 cm piece of white filter paper for oviposition.

Treatments were assigned randomly within each row or block. All ovitraps received 100 ml of water from a container found behind an equipment storage building on site, containing mosquito larvae (conditioned water). No eggs or larvae were transferred to the test cups. Two ovitraps within the block received 50 µl of Vectobac 12 AS® (LOT # 65-841-BA, 1.2% a.i, Valent BioSciences™ Libertyville, IL). This dose of B.t.i. is lethal to *Ae. albopictus* larvae (unpublished data). A single high dose, instead of an increasing concentration of doses, was used to establish if B.t.i. influences oviposition behavior. To test whether the effect of B.t.i. applications increase or decrease over time, filter papers were collected after 24 h and replaced with new filter papers over a 72 h period after placement in the field. Papers were brought to the laboratory and eggs were counted with the aid of a dissecting microscope. After 72 h, the liquid in the containers was examined for larvae and eggs and the clear plastic cups and water were discarded. Each run of the experiment received fresh conditioned water, new filter papers, and unused plastic cups.

Oviposition experiments were performed in an indoor environmental chamber maintained at 27 ºC on a 12h:12h light:dark cycle. Landing adult female *Ae. albopictus* were collected in the field with a mechanical aspirator. Four female *Ae. albopictus* were placed in a collapsible rearing cage (61cm x 61cm x 61cm). Two separate cages with four female *Ae. albopictus* each were used for each run of the experiment. After the mosquitoes were released into the cage, two ovitraps made of the same components that were used in the field experiments were placed in each cage. Before placement into the cage, the control ovitrap received only 100 ml of water, and the treatment ovitrap received 50 µL of Vectobac 12 AS® and 100 ml of water. Each cage received one treatment and one control ovitrap. To reduce placement effect, the cage was divided into four quadrants of equal size and the ovitraps were randomly assigned to a quadrant, with one ovitrap occupying one quadrant. This experiment was repeated five times with tap water filtered through a PUR® CT-5000 L filter. For three repetitions, 100 ml of conditioned water was used. Filter papers were collected after 72 h. Papers were brought into the laboratory and eggs were counted.

A mixed procedure ANOVA tested whether differences in the number of eggs between the two treatments, the day of sampling, and interaction of treatment and day variables were significant (SAS ver. 8, SAS Institute Inc., Cary NC). Due to the small sample size of the laboratory experiment ($n < 6$), Mann-Whitney U tests were used to test for a statistically significant difference between the number of eggs laid in the treatment and control ovitraps (Minitab ver. 14, Minitab Inc., State College PA).

For the laboratory experiments, an oviposition activity index (OAI) was calculated using the formula:

$$\mathrm{OAI} = (N_{\mathrm{Treatment}} - N_{\mathrm{Control}})/(N_{\mathrm{Treatment}} + N_{\mathrm{Control}})$$

where N is the number of eggs laid in each treatment (Kramer et al. 1979).

All calculated values fall between 1.0 and -1.0. Values of zero indicate no difference between treatments, but if values of greater than zero are found, it indicates that there has been no avoidance of oviposition in the containers with B.t.i. (Allan and Kline 1998). This index is only a coarse measurement of whether or not the females are influenced by a particular treatment. Only the endpoint (actual number of eggs laid) is measured, which ignores all potential pre-egg laying behaviors (Kramer et al. 1979).

RESULTS

Thirteen replicates of the field experiment were completed from 20 May through 18 September 2002. Mosquitoes laid eggs on the filter papers in one or more of the ovitraps in all 13 trials. Eggs were found on papers in both the treated and untreated ovitraps. The most eggs laid in a single ovitrap that contained B.t.i. was 39, and for the control ovitraps the highest number was 28. Even though other container-breeding mosquitoes were present at the Cherry Farm Insectary (Richardson et al. 1995), all mosquitoes reared from collected eggs were *Ae. albopictus*. Voucher specimens were placed in the Clemson University Arthropod Collection.

The mean (±SE) number of eggs per ovitrap and minimum and maximum numbers of eggs per sampling period for all sampling periods are given in Table 1. A total of 1,687 eggs was laid during the 13 trials. Ovitraps that contained B.t.i. received 939 eggs and control ovitraps received 748 eggs. When the number of eggs in treated and control ovitraps for the 24-h period were combined for all 13 trials, 290 eggs were laid in the ovitraps that contained B.t.i. and 311 eggs were laid in the control ovitraps. For the 48-h period, 418 eggs were laid in the B.t.i. ovitraps and 216 in the control ovitraps. During the 72-h period, 231 eggs were laid in the B.t.i. ovitraps and 184 in the control ovitraps.

Using the mixed procedure ANOVA, no significant differences were found in the number of eggs laid in the ovitraps for the two treatments ($F_{1,3}$ =0.70, P=0.4647), day ($F_{2,18}$ =0.50, P=0.6126) or day-treatment interaction ($F_{2,18}$ =2.12, P=0.1486). No significant differences were found in the treatment or day-treatment interaction for any of the 13 trials individually. In trials 1 ($F_{2,30}$ =12.43, P=0.0001), 6 ($F_{2,22}$ = 4.92, P= 0.0172) and 11 ($F_{2,28}$ = 3.67, P = 0.0383) a significantly different number of eggs were laid during the 48-h period versus the 24- and 72-h intervals; however the difference was not related to the treatment variable.

In the cage experiments with *Ae. albopictus* and filtered tap water, a total of 752 eggs was laid over the five trials. When the number of eggs was combined across the five trials, the ovitraps containing B.t.i had a total of 662 eggs (88%) versus 90 eggs (22%) laid in the control ovitraps. When OAIs were calculated for each trial all were positive (1.00, 0.95, 0.67, 0.36, 0.85). Over the five trials, there were significantly more eggs laid in the containers that had B.t.i. versus those without B.t.i. (P= 0.0119; Mann-Whitney U test).

For the cage experiment that used conditioned water, a

Table 1. Average number of eggs ± the standard error (minimum - maximum) deposited in ovitraps that did and did not contain 50 µl of *Bacillus thuringiensis* var. *israelensis* in 100 ml of conditioned water over a 72-hour period from 20 May through 18 September 2002. Treatments marked N/A were not collected due to loss of ovitrap.

	24 h		48 h		72 h	
	B.t.i.	Control	B.t.i.	Control	B.t.i.	Control
1	4.6 ±1.3 (0-11)	5.8 ±2.2 (0-19)	0.1±0.1 (0-1)	0.8±0.8 (0-6)	0.5±0.5 (0-4)	0.3±0.3 (0-2)
2	0.0±0.0 (0-0)	2.3±2.1 (0-17)	3.3±1.5 (0-11)	3.0±1.6 (0-13)	0.3±0.3 (0-7)	4.8±2.2 (0-15)
3	3.4±1.4 (0-12)	7.8±1.4 (0-15)	N/A N/A	N/A N/A	N/A N/A	N/A N/A
4	1.5±0.7 (0-10)	2.9±1.1 (0-8)	N/A N/A	N/A N/A	N/A N/A	N/A N/A
5	4.8±1.5 (0-10)	2.5±0.8 (0-6)	8.8±3.5 (0-24)	2.3±1.3 (0-9)	1.9±3.5 (0-6)	0.5±0.3 (0-2)
6	1.3±0.1 (0-8)	4.0±1.5 (0-11)	6.8±3.5 (0-22)	3.4±1.4 (0-9)	1.0±1.0 (0-5)	0.0±0.0 (0-0)
7	3.6±2.5 (0-20)	1.3±0.9 (0-7)	9.8±3.0 (0-20)	3.7±1.7 (0-11)	N/A N/A	N/A N/A
8	5.5±3.3 (0-28)	6.0±3.1 (0-24)	6.7±6.1 (0-37)	1.0±0.6 (0-3)	9.8±3.7 (0-16)	6.3±4.0 (0-17)
9	2.5±2.5 (0-20)	3.8±1.2 (0-8)	3.4±2.7 (0-22)	1.7±1.7 (0-9)	7.0±2.1 (0-16)	5.8±2.6 (0-15)
10	6.3±1.8 (0-12)	3.6±1.6 (0-10)	6.3±2.2 (0-18)	3.8±1.0 (0-8)	6.4±3.6 (0-30)	3.6±1.1 (0-9)
11	2.6±1.4 (0-10)	2.3±1.0 (0-9)	7.1±3.3 (0-30)	8.3±3.1 (0-28)	2.7±0.9 (0-6)	3.6±1.0 (0-9)
12	1.3±0.1 (0-8)	0.3±0.2 (0-1)	0.0±0.0 (0-0)	0.6±0.6 (0-4)	N/A N/A	N/A N/A
13	0.4±0.4 (0-3)	0.9±0.4 (0-2)	5.5±4.8 (0-39)	1.9+1.1 (0-3)	4.1±1.8 (0-15)	4.3±1.2 (0-9)

total of 265 eggs were laid over three trials. When the number of eggs for the three trials were combined, 214 (81%) eggs were laid in the B.t.i.- containing ovitraps and 51 (19%) were laid in the control ovitraps. Only trials two and three had enough eggs to compute an OAI. For trial two, the OAI was 0.9 and for trial three it was 0.18. Over the three trials there was not a statistically significant difference between the number of eggs laid in the treatments versus the controls (*P*=0.6625; Mann-Whitney U test).

DISCUSSION

In both field and laboratory experiments, female mosquitoes did not avoid ovipositing in ovitraps that contained B.t.i. Over the 13 weeks of the field experiment, more eggs were laid in B.t.i.-containing ovitraps than in the controls, although there was no statistically significant difference between treatment and control. In the laboratory, more eggs were laid in the ovitraps containing B.t.i. than the controls and the OAIs were all >0, indicating that the females did not avoid ovipositing in ovitraps with water containing B.t.i. There was also a significant difference in the number of eggs laid between the two treatments in the filtered tap water trials.

In both laboratory and field trials using conditioned water, there was no significant difference in the number of eggs laid in the treatment and control ovitraps. But due to differences in experimental design, it is difficult to directly compare results of the field and laboratory experiments. In both cases, however, when the number of eggs is totaled over all of the trials, a greater number of eggs was laid in the ovitraps with B.t.i. than in the controls.

Oviposition behavior in mosquitoes is governed by several factors such as pheromones, water chemical composition, and presence of pathogens and predators (Bentley and Day 1989). An oviposition attractant pheromone has not been found in *Ae. albopictus* (Allan and Kline 1998). Because such a strong reaction to B.t.i. has been found in the laboratory, potentially visual or chemical stimuli from the water might have played a more important role than in the field (Allan and Kline 1998). Ovipositing female *Aedes albopictus* are attracted to dark-colored water (Gubler 1971). The high concentration used for the applications of B.t.i. darkened both the conditioned and filtered water. In the closed laboratory environment, this visual stimulus might have been a factor in oviposition site selection, not the presence of B.t.i. or any other chemical (Trexler et al. 2003). In fact, very few chemicals have been found to attract ovipositing *Ae. albopictus* (Trexler et al. 2003).

These results indicate that a program dispersing B.t.i. by ULV would not influence the oviposition behavior of *Ae. albopictus* in South Carolina. Additional experiments to determine a color effect, using artificial nontoxic dye, and the testing of the individual components of Vectobac 12 AS® at various concentrations would have to be completed before strong conclusions could drawn. If this technique is used on isolated populations, selection over time for behavioral avoidance of containers treated with B.t.i is possible.

Acknowledgments

I thank W. C. Bridges for help with the statistics and P. H. Adler, M. J. Bangs, and A. G. Wheeler Jr. for helpful reviews of the manuscript. The views expressed in this article are those of the author and do not reflect the official policy or position of the Navy, Department of Defense, or the U. S. Government.

REFERENCES CITED

Allan, S. A. and D. L. Kline. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 35: 943-947.

Bentley, M. D. and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annu. Rev. Entomol. 34: 401-421.

Gubler, D.J. 1971. Studies on the comparative oviposition behavior of *Aedes* (*Stegomyia) albopictus* (Skuse) and *Aedes (Stegomyia) polynesiensis* Marks. J. Med. Entomol. 8: 675-682.

Kramer, W.L., Y.S. Hwang, and M.S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8: 1111-1117.

Lee, H.L., E.R. Gregorio, M.S. Khadri, and P. Seleena. 1996. Ultralow volume application of *Bacillus thuringiensis* ssp. *israelensis* for the control of mosquitoes. J. Am. Mosq. Contr. Assoc. 12: 651-655.

Martin, P.A.W. and R.S. Travers. 1989. World-wide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55: 2437-2442.

Moore, C.G., D.B. Francy, D.A. Eliason, and T.P. Monath. 1988. *Aedes albopictus* in the United States: rapid spread of a potential disease vector. J. Am. Mosq. Contr. Assoc. 4: 356- 361.

Nasci, R.S., G.B. Wright, and F. S. Willis. 1994. Control of *Aedes albopictus* larvae using time-release larvicide formulations in Louisiana. J. Am. Mosq. Contr. Assoc. 10: 1-6.

Richardson, J.H., W.E. Barton, and D. C. Williams. 1995. Survey of container-inhabiting mosquitoes in Clemson, South Carolina, with emphasis on *Aedes albopictus*. J. Am. Mosq. Contr. Assoc. 11: 395-400.

Trexler, J.D., C.S. Apperson, C. Gemeno, M.J. Perich, D. Carlson, and C. Schal. 2003. Field and laboratory evaluations of potential oviposition attractants for *Aedes albopictus* (Diptera: Culicidae). J. Am. Mosq. Contr. Assoc. 19: 228-234.

Yap, H.H., S.C. Chong, C.R. Adanan, N.L. Chong, B. Rohaizat, Y. Abdul Malik, and S. Y. Lim. 1997. Performance of ULV formulations (Pesguard™ 102/ Vectobac™ 12 AS) against three mosquito species. J. Am. Mosq. Contr. Assoc. 13: 384-388.